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Simultaneous Transition Metal Catalytic Oxidation and Enzymatic Reduction using Orthogonal Reagents

Francesco G. Mutti, Andreas Orthaber, Joerg H. Schrittwieser, Johannes G. de Vries, Rudolf
Pietschnig, and Wolfgang Kroutil*

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S1. General information

Chemicals and Enzymes: (*S*)-2-chloro-1-phenylethanol (**2a**), chloroacetophenone (**3a**), acetone, chloroacetone, potassium tert-butoxide and sodium formate were purchased either from Sigma-Aldrich or Acros. The iridacycle catalyst (**1**) was synthesised as reported in literature,¹ starting from commercially available [Cp*IrCl₂]₂ (Sigma-Aldrich), potassium hexafluorophosphate, and N-methylbenzylamine. Racemic 2-chloro-1-phenylethanol (**2a**), 1-phenoxy-3-chloroethanol (**2b**), 1-chloro-2-octanol (**2c**)² and 6,6-dimethyl-2-chlorocyclohexanone (**3f**)³ were synthesised according to literature. Toluene was dried with 3 Å molecular sieves. Distilled water was used in the preparation of all aqueous buffers.

ADH-A from *Rhodococcus ruber* was overexpressed in *E. coli* BL23(DE3)/pET22b as previously reported,⁴ the cells were disrupted and enzyme was partially purified by heat treatment (60°C, 20 min; then centrifugation). NAD-specific formate dehydrogenase 002 from *Candida boidinii* and β-NADH disodium salt were purchased from Codexis.

All GC analyses were carried out with an Agilent 7890 A GC system, equipped with standard FID detector and using a Chrompack Chirasil Dex-CB column (25m, 320 μm, 0.25 μm). Helium was used as carrier gas and EtOAc was used as solvent.

The following sterical demanding α-haloketones were investigated as hydrogen acceptors: 3-chloro-2-norbornanone, 2-chlorocyclopentanone, 2-chlorocyclohexanone, 2-8-dichlorocyclooctanone, 2-bromocyclohexanone, 2-chloro-α-tetralone, 6,6-dimethyl-2-chlorocyclohexanone **3f**.

S2. Racemisation experiment

a) in toluene: Iridacycle (**1**) (2.11 mg, 3.33 μmol) and KO^tBu (0.45 mg, 4.00 μmol) were placed into a 2 mL vial with cap. After everything was transferred into a glove-box, dry

toluene (1.0 mL) was added. The solution became shining purple within 15 minutes, indicating activation of the catalyst. Finally, the substrate (*S*)-2-chloro-1-phenylethanol (**2a**) (8.81 μ L, 66 μ mol) was added and the vial was thoroughly closed. The sample was shaken at 1000 rpm in an Eppendorf thermomixer in vertical position, at 21°C for 1 h. The sample was filtered using Rotilabo[®]-Spritzenfilter (13 mm, 0.2 μ m, Nylon) and analysed by GC.

b) in toluene / aqueous buffer: **1** (2.11 mg, 3.33 μ mol) was activated in dried toluene (300 μ L) as described above in a glove-box. Then distilled water (700 μ L) was put into a second vial and the organic solution containing the activated catalyst was gently pipetted onto the aqueous phase. Finally, the substrate (*S*)-**2a** (8.81 μ L, 66 μ mol) was added and the vial was thoroughly closed. Samples were vigorously shaken for 2 h at 21°C. The reaction was stopped by extraction with ethyl acetate (2 \times 500 μ L) and the organic layer was separated from the aqueous phase by centrifugation (2 min, 13000 rpm) and dried with Na₂SO₄. Conversion was determined by GC analysis.

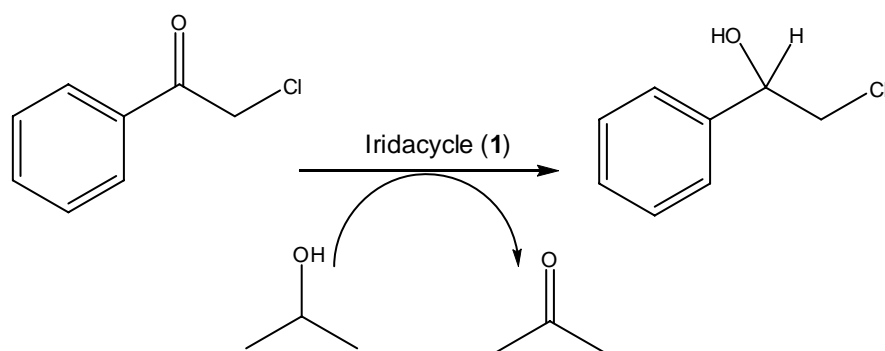
S3. Oxidation of racemic chlorohydrins with iridacycle catalyst

1 (2.11 mg, 3.33 μ mol, 5 mol% or 0.85 mg, 1.33 μ mol, 2 mol%) and KOtBu (0.45 mg, 4.00 μ mol or 0.18 mg, 1.6 μ mol) were placed into a 2 mL vial with cap. Then, everything was transferred into a glove-box and dry toluene (1 mL) was added. The solution became shining purple within 15 minutes, indicating activation of the catalyst. Then, the oxidant [e.g. acetone (**3d**), chloroacetone (**3e**), 6,6-dimethyl-2-chlorocyclohexanone (**3f**)] and the substrate (*S*)-**2a** (8.81 μ L, 66 μ mol) were added. The vial was thoroughly closed and reaction was run at 21°C. Maximum conversion is reached after approximately 3 h. The sample was filtered using Rotilabo[®]-Spritzenfilter (13 mm, 0.2 μ m, Nylon) and analysed by GC.

S4. Reduction of chloroacetophenone with iridacycle catalyst

The aim of this experiment is to prove that **1** can reduce ω -chloroacetophenone (**3a**) in

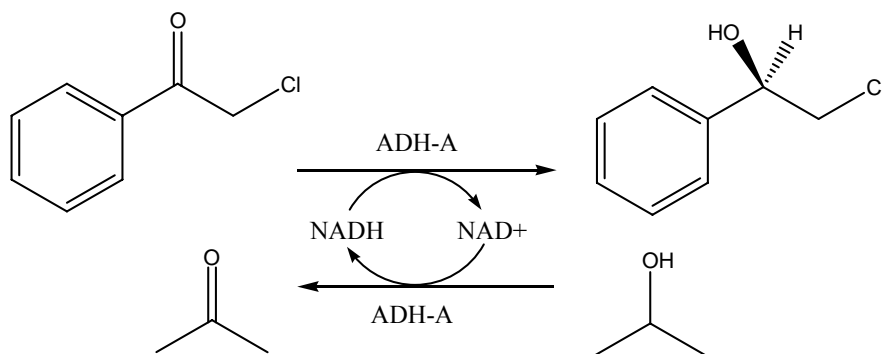
combination with a reducing reagent such as 2-propanol.



1 (2.11 mg, 3.33 μ mol, 5 mol%) was activated with KOtBu (0.45 mg, 4.00 μ mol) in dry toluene (200 μ L) in a glove-box by stirring for 15 minutes at 21°C. The reaction was carried out in a 2 mL dark glass vial filled with aqueous buffer Tris-HCl (700 μ L, pH 7.5, 50 mM). The organic phase containing the activated catalyst was transferred onto the aqueous solution and 2-propanol (200 μ L) and substrate acetophenone (**3a**) (10.2 mg, 66 μ mol) were added. The reaction was run for 22 hours at 21°C at 1000 rpm on an Eppendorf thermomixer kept in vertical position. The reaction was stopped by extraction with ethyl acetate (2×500 μ L) and the organic layer was separated from the aqueous phase by centrifugation (2 min, 13000 rpm) and dried with Na₂SO₄. Conversion was determined by GC analysis.

S5. Reduction of ω -chloroacetophenone **3a** with ADH-A

The activity of the ADH-A preparation was tested in the reduction of chloroacetophenone **3a**:

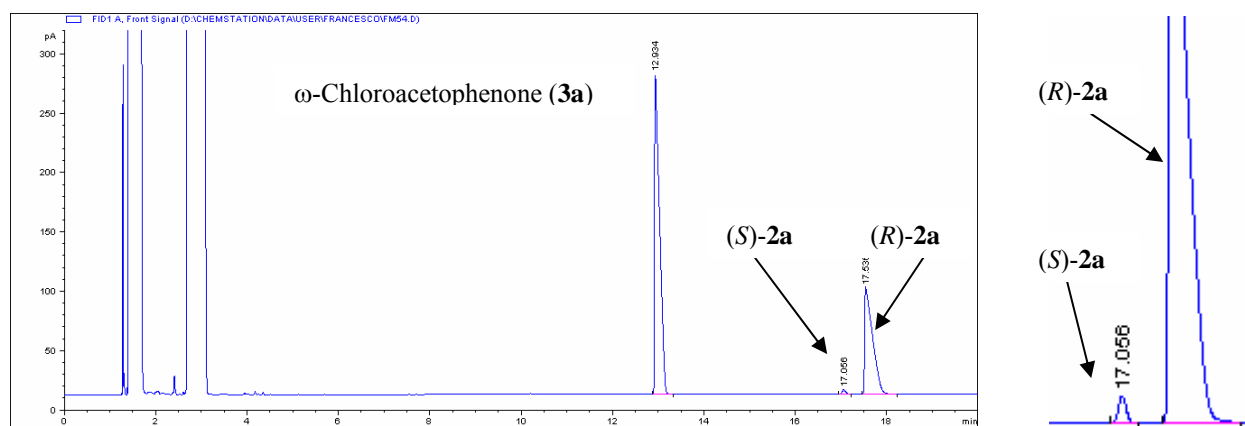


ADH-A preparation (500 μ L, 25 U) and a solution of NADH (300 μ L) in buffer (Tris-HCl, pH 7, 50 mM) were mixed to give a NADH concentration 1 mM. Then substrate **3a** (10.2 mg,

66 μmol) and 2-propanol **2d** (200 μL) were added. Reactions were shaken at 30 $^{\circ}\text{C}$ for 24 h at 120 rpm on a rotary shaker plate and stopped by extraction with ethyl acetate ($2 \times 500 \mu\text{L}$). The organic layer was separated from the aqueous phase by centrifugation (5 min, 13000 rpm) and dried with Na_2SO_4 . Conversion was determined by GC. Quantitative conversion and perfect e.e. (> 99%) was obtained within 24 hours.

S6. Reduction of ω -chloroacetophenone **3a with ADH-A in the presence of iridacycle **1****

a) NADH recycling with 2-propanol: a stock solution of activated **1** (2.11 mg, 3.33 μmol , 5 mol%) was prepared by treatment with KOtBu (0.45 mg, 4.00 μmol) in dry toluene (200 μL) in a glove-box by stirring for 15 minutes at 21 $^{\circ}\text{C}$. A 0.5 mol% solution of **1** was prepared by dilution with toluene. A solution of ADH-A in buffer (Tris-HCl, pH 7.5, 50 mM, 450 μL , 500 U) was mixed with a NADH solution in buffer Tris-HCl (pH 7.5, 50 mM, 150 μL) to reach NADH concentration 4 mM. The organic phase containing **1** was poured onto the aqueous phase containing ADH-A and NADH. Then, 2-propanol (200 mL) and substrate **3a** (10.2 mg, 66 μmol) were added. The reaction was run for 1 hour at 30 $^{\circ}\text{C}$ on a rotary shaker plate at 250 rpm and stopped by extraction with ethyl acetate ($2 \times 500 \mu\text{L}$). The organic layer was separated from the aqueous phase by centrifugation (5 min, 13000 rpm) and dried with Na_2SO_4 . The reduction proceeded with 35% conversion and 96% e.e. The GC chromatogram is depicted below:



b) NADH recycled with FDH-sodium formate: ADH-A (500 μ L, 500 U) in Tris-HCl buffer (pH 7.5, 50 mM) was mixed with FDH (1 mL, 550 U). NADH (5.7 mg, 4 mM) and sodium formate (18 mg, 265 μ mol) were added. A toluene solution of activated **1** (500 μ L, 1 mol%) was poured onto the aqueous phase and **3a** was added (5.1 mg, 33 μ mol). The reaction was shaken on a rotary plate at 120 rpm, for 4 h, at 30°C and stopped by extraction with ethyl acetate ($2 \times 500 \mu$ L). The organic layer was separated from the aqueous phase by centrifugation (5 min, 13000 rpm) and dried with Na_2SO_4 . The reduction proceeded with 82% conversion and 97% e.e.

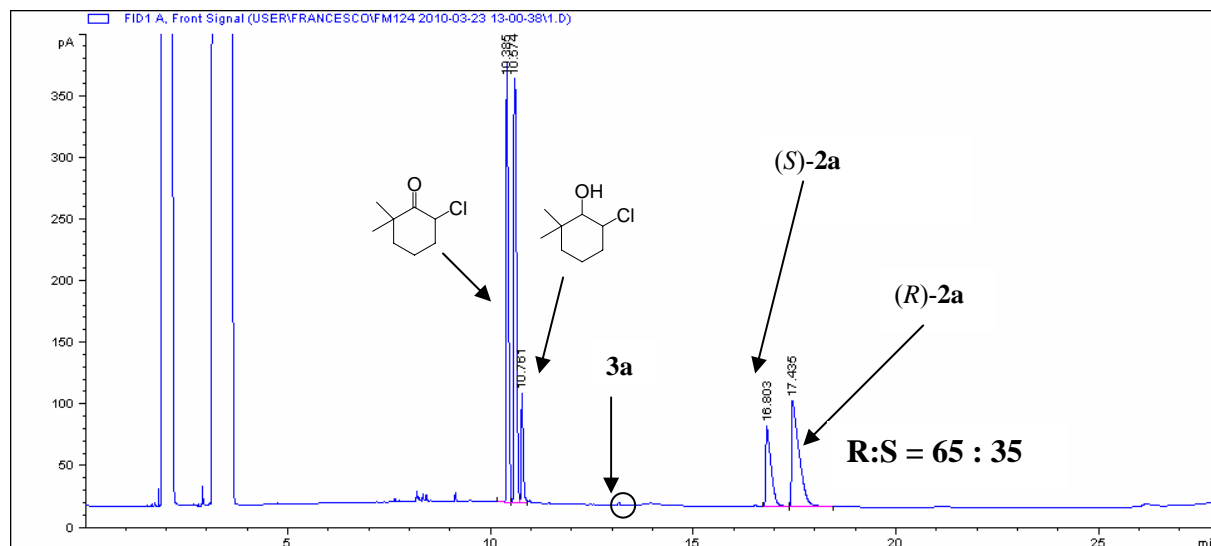
S7. Combination of iridacycle catalyst and ADH-A for simultaneous oxidation – reduction

Solutions of ADH-A (500 U, 500 μ L, pH 7.5, 50 mM), FDH (140 μ L, 30 U) and NADH (110 μ L, final concentration 1 mM) in Tris-HCl buffer were combined. A toluene solution of activated **1** (150 μ L; 2 mol % or 5 mol%) was poured onto the aqueous phase. Then, a toluene solution of 2,2-dimethyl-6-chloro cyclohexanone (**4**) (150 μ L,) was added. The amount of oxidant was varied from 2 eq. (66 μ mol, 10.6 mg), 5 eq. (165 μ mol, 26.5 mg) to 10 eq. (330 μ mol, 53.0 mg). Finally, the substrate *rac*-**2a** (4.4 μ L, 33 μ mol) or *rac*-**2b** (5.1 μ L, 33 μ mol) or *rac*-**2c** (7 μ L, 33 μ mol) was added. Reactions were shaken on a rotary plate at 120 rpm for 16 h at 30°C and stopped by extraction with ethyl acetate ($2 \times 500 \mu$ L). The organic layer was

separated from the aqueous phase by centrifugation (5 min, 13000 rpm) and dried with Na_2SO_4 .

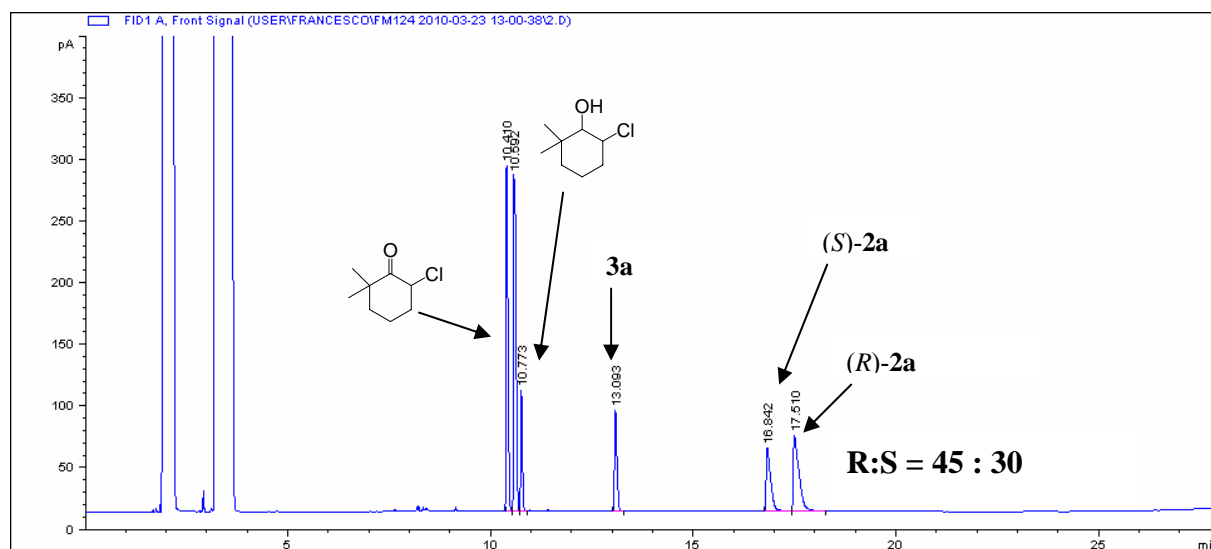
Some typical chromatograms are shown below:

(1) **1** (2 mol%), **4** (2 eq.), substrate **2a**, reaction mixing by vigorously shaking.



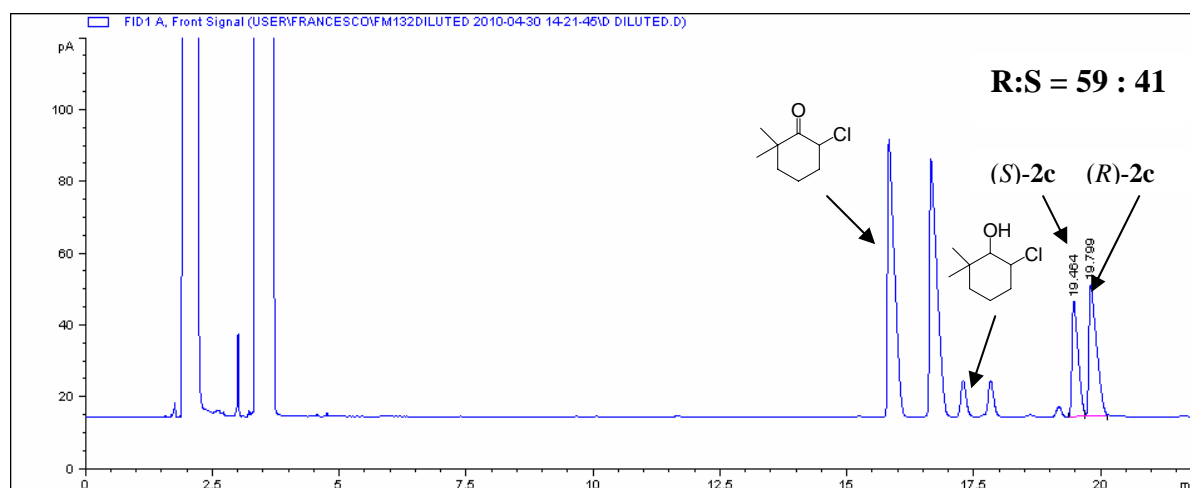
Starting from racemic **2a**, enantioenriched mixture of the (*R*)-enantiomer (R:S = 65:35; e.e.= 30%) was obtained. The amount of remaining intermediate chloroacetophenone **3a** was negligible.

(2) **1** (2 mol%), **4** (2 eq.), substrate **2a**, reaction mixing by gentle stirring, maintaining 2-phase system.



Starting from racemic **2a**, we obtained an enantioenriched mixture of the (*R*)-enantiomer (R:S = 45:30; e.e.= 20%). The amount of remaining intermediate chloroacetophenone **3a** was herer significant (26%) (see main text). Therefore, when the reaction is performed with phases separated, the diffusion of **3a** from the organic phase to the aqueous phase is the rate limiting step. The diffusion is faster, when the reaction is shaken and no phases separation is observed (exp. 1).

(3) **1** (2 mol%), **4** (2 eq.), substrate **2c**, reaction mixing by shaking



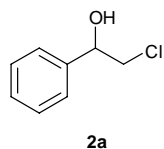
Starting from racemic 1-chloro-2-octanol (**2c**), we obtained an enantioenriched mixture of the Prelog R enantiomer (R:S = 59:41; e.e.= 17%). The amount of remaining intermediate 1-chloro-2octanone (**3c**) was negligible.

S8. Analytics

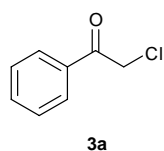
Method A: GC program parameters; injector 200°C; constant flow 1.5 mL/min.; temperature program 60°C/hold 1 min.; 140°C/rate 10°C per min/hold 15 min; 180°C/rate 10°C per min./hold 0 min.

Method B: GC program parameters; injector 200°C; constant flow 1.5 mL/min; temperature program 100°C/hold 1 min.; 145°C/rate 1°C per min/hold 0 min; 180°C/rate 10°C per min/hold 0 min.

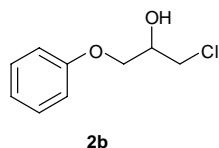
Method C: GC program parameters; injector 200°C; constant flow 1.5 mL/min; temperature program 60°C/hold 1 min; 110°C/rate 10°C per min/hold 0 min; 124°C/rate 0.5°C per min/hold 0 min; 180°C/rate 10°C per min/hold 0 min.



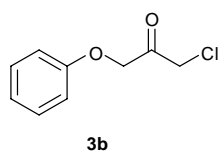
GC/Method A: (*S*)-**2a** 16.9 min.; (*R*)-**2a** 17.5 min.; the absolute configuration was assigned by comparison of elution order on GC and coinjection with commercially available (*S*)-**2a** and (*R*)-**2a**.



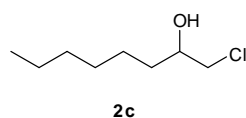
GC/Method A: **3a** 12.9 min.; the retention time was compared to commercially available **3a**.



GC/Method B: (*R*)-**2b** 48.7 min.; (*S*)-**2b** 48.9 min.; the absolute configuration was assigned by the comparison of elution order on GC with chlorohydrins previously obtained.⁵



GC-method A: **3b** 19.8 min; reference compound was obtained according to literature.⁵



GC/Method C: (*S*)-**2c** 19.5 min.; (*R*)-**2c** 19.8 min.; the absolute configuration was assigned by the comparison of elution order on GC with chlorohydrins previously obtained.⁵

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